

WHAT IS CLAIMED IS:

1. A method for determining an analyte in a sample using an analytical element, the method comprising:

5 providing a mixture by contacting the sample with a binding partner 2 of a specific binding pair 1 (partner 2 of pair 1), and a binding partner 2 of a specific binding pair 2 (partner 2 of pair 2),

adding the mixture to a sample application zone of the analytical element, wherein the element comprises a material enabling liquid transport between the sample application zone and
10 a detection zone located downstream thereof, wherein the detection zone comprises a binding partner 1 of specific binding pair 1 (partner 1 of pair 1) immobilized in such a manner that it is able to bind to the partner 2 of pair 1, and wherein a labeled partner 1 of specific binding pair 2 (partner 1 of pair 2) is present upstream of the detection zone and impregnated on the material such that it can be detached by liquid and is able to bind to the partner 2 of pair 2, and

15 detecting the presence or absence of the label in the detection zone, thereby determining the analyte in the sample.

2. The method of claim 1 wherein the specific binding pair 1 and the specific binding pair 2 independently comprise a pair of specific binding partners selected from the group consisting of
20 a hapten and an antibody, an antigen and an antibody, a lectin and a sugar/saccharide, a ligand and a receptor, avidin/streptavidin and biotin, a nucleic acid and a nucleic acid.

3. The method of claim 1 wherein the partner 1 of pair 2 is an antibody against the partner 2 of pair 2.

4. The method of claim 3 wherein the partner 1 of pair 2 is an antibody against digoxigenin or digoxin.

5 5. The method of claim 1 wherein the partner 1 of pair 2 is labeled with an enzyme or direct label.

6. The method of claim 5 wherein metal or latex particles are used as the direct label.

10 7. The method of claim 1 wherein the partner 1 of pair 2 is located in the sample application zone.

8. The method of claim 5 wherein the partner 1 of pair 2 is located in the sample application zone.

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9. The method of claim 1 wherein an antibody for specific binding with a preselected antigen or hapten is conjugated with the partner 2 of pair 1 and the antibody is conjugated with the partner 2 of pair 2.

20 10. The method of claim 1 wherein an antigen, hapten or oligopeptide is conjugated with the partner 2 of pair 1 and the antigen, hapten or oligopeptide is conjugated with the partner 2 of pair 2, wherein the antigen, hapten or oligopeptide specifically binds to a preselected antibody.

11. The method of claim 1 wherein the partner 2 of pair 1 and the partner 2 of pair 2 are present in separate containers, wherein the separate containers do not include the analytical element.
- 5 12. The method of claim 1 wherein the partner 2 of pair 1 and the partner 2 of pair 2 are stored together in one container, wherein the container does not include the analytical element.
13. The method of claim 1 wherein the partner 2 of pair 1 is conjugated to a nucleotide, oligonucleotide, a nucleic acid, an antibody, a hapten or antigen or an epitope representing an
10 antigen or a lectin or a receptor for a ligand.
14. The method of claim 13 wherein the partner 2 of pair 1 is biotin.
15. The method of claim 1 wherein the partner 2 of pair 2 is conjugated to a nucleotide,
15 oligonucleotide, a nucleic acid, an antibody, a hapten or antigen or an epitope representing an antigen or a lectin or a receptor for a ligand.
16. The method of claim 15 wherein the partner 2 of pair 2 is a hapten.
- 20 17. The method of claim 16 wherein the hapten is digoxigenin or digoxin.
18. A method for determining the presence of an analyte using an analytical element comprising a material enabling liquid transport between a sample application zone where a

sample is applied and a detection zone located downstream thereof, wherein the detection zone comprises a binding partner 1 of specific binding pair 1 (partner 1 of pair 1) immobilized in such a manner that it is able to bind to a binding partner 2 of specific binding pair 1 (partner 2 of pair 1), and wherein a labeled partner 1 of specific binding pair 2 (partner 1 of pair 2) is present upstream of the detection zone and impregnated on the material such that it can be detached by liquid and is able to bind to a specific binding partner 2 of specific binding pair 2 (partner 2 of pair 2); the method comprising:

adding to the sample the sample application zone a substance derived from and representing the analyte wherein the substance comprises partner 2 of pair 1 and partner 2 of pair 2,

moving said substance by liquid transport in the analytical element towards the detection zone; and

binding said substance to partner 1 of pair 1 in the detection zone; and
detecting the labelled partner 1 of pair 2 bound in the detection zone.

19. The method of claim 18 wherein the substance derived from and representing the analyte is formed by adding to the analyte an antibody wherein part of the antibody carries partner 2 of pair 1 and the other part of the antibody carries partner 2 of pair 2.

20. The method of claim 18 wherein the substance derived from and representing the analyte is formed by adding to the analyte an antigen, hapten or oligopeptide wherein a part of the antigen, hapten or oligopeptide carries partner 2 of pair 1 and the other part of the antigen, hapten or oligopeptide carries partner 2 of pair 2.

21. The method of claim 18 wherein the analyte is a nucleic acid which is amplified, whereby partner 2 of pair 1 or partner 2 of pair 2 is bound to a nucleotide or to an oligonucleotide that is incorporated into the amplification product of said nucleic acid, and the amplification product is hybridized with a complementary nucleic acid having partner 2 of pair 1 or partner 2 of pair 2 bound thereto, provided that when the amplification product has partner 2 of pair 1 bound thereto, the complementary nucleic acid has partner 2 of pair 2 bound thereto and when the amplification product has partner 2 of pair 2 bound thereto, the complementary nucleic acid has partner 2 of pair 1 bound thereto.

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22. The method of claim 18 wherein the analyte is a nucleic acid and said substance comprises the nucleic acid hybridized with two nucleic acid probes one of which contains partner 2 or pair 1 and the other contains partner 2 of pair 2.

15 23. An analytical element for determining the presence of an analyte, the element consisting essentially of a material enabling liquid transport between a sample application zone where a sample is applied and a detection zone located downstream thereof, wherein the detection zone contains a partner 1 of a specific binding pair 1 (partner 1 of pair 1) immobilized in such a manner that it is able to bind to a partner 2 of the specific binding pair 1 (partner 2 of pair 1) when the partner 2 of pair 1 contacts the partner 1 of pair 1, wherein the partner 2 of pair 1 is not the analyte, and wherein a labeled partner 1 of a specific binding pair 2 (partner 1 of pair 2) is present upstream of the detection zone and impregnated on a material such that it can be detached by liquid and is able to bind to a partner 2 of the specific binding pair 2 (partner 2 of

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pair 2) when the partner 2 of pair 2 contacts the partner 1 of pair 2, and the partner 2 of pair 2 is not the analyte, wherein both the partner 2 of pair 1 and the partner 2 of pair 2 are (i) not impregnated or immobilized on the material and (ii) added to the sample to bind the analyte before the sample is applied to the application zone.